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13. ABSTRACT (Maximum 200 Words)

The purpose of these studies is to elucidate the pharmacogenetic factors that contribute to variation in human response to tamoxifen (TAM) and raloxifene (RAL). We had previously identified and partially characterized common genetic polymorphisms in two human drug-metabolizing genes, SULT1A1 and UGT1A6. We hypothesized that these polymorphisms contributed to variation in TAM or RAL metabolism. These studies were divided into three aims with the purpose of 1) biochemically characterizing the contribution of these enzymes to the metabolism of TAM and RAL; 2) developing cell model systems to study allele-specific differences in cellular response to these molecules and; 3) perform a clinical pharmacogenetic study to evaluate the association of common genetic polymorphisms in drug metabolizing genes with variable clinical response to TAM. Thus far we have determined that SULT1A1 and UGT1A6 contribute to the inactivation of 4-hydroxytamoxifen (OHT), the active metabolite of TAM, and that a separate enzyme, UGT1A9 catalyzed the glucuronidation of RAL. We have determined genotype/phenotype correlation for UGT1A6 alleles in a bank of human liver tissue and have generated HEK 293 cell lines that stably express each of the four UGT1A6 allozymes. The UGT1A6*2 allozyme, when expressed homozygously, is associated with high UGT1A6 activity. We established MCF-7 breast cancer cell lines stably expressing the wildtype and variant SULT1A1 alleles and have measured allele-specific differences in the response of these cells to estrogens and OHT. These studies suggest that pharmacogenetic factors might contribute to variable cellular response to antiestrogenes.

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INTRODUCTION

The goals of this proposal are to elucidate the pharmacogenetic factors that influence cellular response to tamoxifen (TAM) and raloxifene (RAL). Specifically, the work described represents a stepwise approach to the study of genetic polymorphisms in human sulfotransferase (SULT) and UDP-glucuronosyltransferase (UGT) genes. Those studies will progress from basic biochemical studies to the use of cell models and will culminate in a clinical pharmacogenetic study. In the first aim, we proposed to biochemically characterize the capacity of wildtype and variant SULT1A1, SULT1A2 and UGT1A6 proteins to conjugate 4-hydroxytamoxifen (OHT) and raloxifene RAL. The second aim focussed on the development of cell models to study allele-specific differences in cellular response to these antiestrogens. Finally, the third aim will determine the association of genetic polymorphisms in several metabolic pathways with human response to TAM in a clinical setting.

BODY

Specific Aim1. Biochemically characterize the capacity of recombinant wildtype and variant SULT1A1 and UGT1A6 proteins to conjugate OHT and RAL in *in vitro* assays. We proposed four sub aims associated with this aim—each to be completed within the first year of funding. Those included:

1) Generation of recombinant wildtype and variant UGT1A6 allozymes
2) Biochemical characterization of the SULT1A1 allozymes with regard to their capacity to sulfate OHT and RAL.
3) Optimization of the glucuronidation assay.
4) Biochemical characterization of the UGT1A6 allozymes with regard to their capacity to glucuronidate OHT and RAL.

In our previous progress reports we presented data regarding biochemical characterization of SULT1A1 allozymes with OHT as a substrate. We also reported on the association between UGT1A6 alleles and level of enzyme activity in a bank of human liver tissues. We now report the biochemical characterization of recombinant UGT1A6 allozymes. Table 1 depicts Vmax and Km values for the four UGT1A6 allozymes that we identified. These data indicate that the *2 allozyme has an approximate two-fold higher specific activity than does the wildtype *1 allozyme. These data are in close agreement with data generated from a bank of human liver tissue samples.

Table 1   Biochemical parameters for recombinant UGT1A6 allozymes

Allozyme	K_M (µM)	V_{max} (nmol/min/mg)	V_{max}/K_M (ml/min/mg)	Variation from *1 (Ref)
*1	1203 ± 365	35.4 ± 4.11	0.029	Reference
*2	639 ± 152	30.3 ± 2.30	0.047	1.6x
*3	843 ± 249	21.1 ± 2.34	0.024	0.8x
*4	817 ± 137	14.2 ± 0.97	0.017	0.6x

Substrate concentration was 0.050-4.0 mM p-nitrophenol; cosubstrate UDPGA concentration was 4.0 mM. Data are from three separate determinations in three unique clones for each allozyme.

Please note that the data in Table 1 were generated using p-nitrophenol as a substrate. This is a prototypic UGT1A6 substrate that whose UV absorption is altered upon conjugation to glucuronic acid. This property makes this substrate ideal for higher throughput assays that are required for detailed kinetic analyses. We have also evaluated the capacity of these allozymes to glucuornidate OHT. OHT is a substrate for UGT1A6 -- however our analysis has revealed that UGTs1A4 and UGT1A9 turn OHT over much faster than does UGT1A6. Because OHT is a relatively poor substrate for UGT1A6 it was not possible to evaluate the kinetics of that reaction to the level of detail as depicted in Table 1.

Specific Aim 2. Determine the antiestrogen response to OHT and RAL of cells in which wildtype and variant SULT1A1 and UGT1A6 allozymes have been expressed. These studies were designed to evaluate whether allele-specific differences in the response of cells to estrogens and antiestrogens could be measured. Sulfation of E2 and OHT results in inactive molecules (ie., they do not interact with the estrogen receptor). Therefore, we hypothesized that cells expressing the less active SULT1A1*2 allozyme would exhibit an enhanced proliferative response to E2 and OHT because those cells would have lower capacity to inactivate those molecules. There were originally six sub aims associated with this specific aim to be completed in months 9 through 30 of the funding period. In our previous progress report we justified modification of the subaims to the following:

1) Generation of expression constructs for SULTs and UGTs
2) Generation of stably transfected human MCF-7 and HEK 293 cell lines
3) Cell proliferation assays
4) Quantitative RT-PCR of stably transfected MCF-7 cell lines
5) Correlation of UGT1A6 Genotype with phenotype in a bank of human liver tissues

We previously reported completion of subaims 1, 2, 4, and 5. Subaim 3 was completed for the SULT1A1 allozymes. Our data revealed that cells expressing the low activity SULT1A1*2 allele proliferated significantly faster in response to treatment with the estrogen 17β-estradiol (E2) and significantly slower in response to the antiestrogen OHT. This was presumably because the capacity of cells expressing the low activity allozyme to inactivate these compounds was compromised. Further mechanistic analyses revealed that the *2 protein has a cellular half-life three to six -fold shorter than the wildtype protein, depending on the cell type studied (see previous progress reports). We have since evaluated the level of OHT sulfate formed in cells expressing SULT1A1*1 or SULT1A1*2. Our data suggest that OHT sulfate is completely transported to the extracellular media (a novel finding) and that less OHT sulfate is detected from cell culture plates expressing SULT1A1*2 than SULT1A1*1. Collectively our data suggest that a common genetic polymorphism in the human SULT1A1 gene (SULT1A1*2 allele) may be associated with greater estrogenic response of breast epithelial cells but may also be associated with greater antiestrogenic response of those cells to OHT.

We have generated HEK 293 cells stably expressing each of the four UGT1A6 allozymes. We have detected no correlation between response of those cells to OHT and UGT1A6 genotype. This result is likely due to the fact that OHT is a poor substrate for UGT1A6.

Specific Aim 3. Determine the association of SULT1A1, UGT1A6, CYP3A4 and CYP2D6 genotypes with the clinical response of women who are being prescribed TAM. The purpose of this aim is to determine the pharmacogenetic factors that impact clinical response to TAM. Because TAM is subject to several competing metabolic pathways—each of them polymorphic—we expanded the pharmacogenetic scope in this aim to encompass the oxidative metabolic pathways that activate TAM to OHT (CYP3A4 and CYP2D6).

In the last progress report we stated that due to complications with approval of our clinical protocol by the Army, accrual of patients was delayed. Accrual began in October of 2001. However, this timing coincided with release of clinical data suggesting that letrozole, an aromatase inhibitor, was more effective than tamoxifen in the treatment of certain breast cancers. These data have resulted in letrozole becoming the first line choice of antiestrogen therapy in women with metastatic breast cancer. Our current protocol stipulates in the inclusion criteria no concomitant use of other hormonal or antiestrogenic agents. Therefore, we have only accrued two women into our study. We have now amended our protocol to study women taking tamoxifen for the adjuvant treatment of breast cancer. Tamoxifen alone remains first line therapy for these women. This amendment differs from our original protocol in that we originally targeted women receiving tamoxifen in the setting of metastatic breast cancer. We have been granted a "no cost" extension of this grant to complete these studies. The amended protocol is attached and we are separately seeking human subjects approval for this protocol through the proper DOD channels.

KEY RESEARCH ACCOMPLISHMENTS

- Established HEK 293 cell lines stably expressing UGT1A6*1, *2, *3 and *4

- Compared kinetic properties of recombinant UGT1A6 allozymes

- Determined that low activity of the SULT1A1*2 allele is attributed to a combination of lower enzymatic "turnover" of substrate (low Vmax) as well as significantly shorter half life of the SULT1A1*2 protein compared to SULT1A1*1.

- Established expression profile of SULTs in human breast tumors and transformed breast carcinoma cell lines

- Developed TLC assay for evaluating OHT glucuronidation kinetics

- Developed high throughput genotyping assays for CYP3A4 and CYP2D6

REPORTABLE OUTCOMES
Abstracts and Meetings

"UGT Pharmacogenetics, and Specificity of Raloxifene Glucuronidation", oral presentation, Jeffrey Zalatoris, DOD Era of Hope Meeting, Orlando, FL, September 2002.

"Common Alleles of Human Sulfotransferase SULT1A1 are Associated with Altered Biochemical and Cellular Response to Estrogens and Antiestrogens", poster presentation, DOD Era of Hope Meeting, Orlando, FL, September 2002.

"UDP-Glucuronosyltransferase-Specific Glucuronidation Inactivates 4-hydroxytamixfen and Raloxifene", Oral Presentation, 11th North American ISSX Meeting, Orlando, FL October 2002. Drig Metab Rev **34**:7, 2002.

"Pharmacogenetics of Human Sulfotransferases", Symposia Speaker, Annual FASEB meeting, San Diego, CA, April 2003.

"Functional Consequences of Individual Variation in Human SULT1A1 and UGT1A6", Symposia Speaker, *th Eurpoean ISSX Meeting, Dijon, France, April 2003.

Manuscripts

Jeffrey J. Zalatoris and Rebecca Blanchard Raftogianis, Association of human UGT1A6 haplotype with level of enzyme activity in human liver tissue, in revision, *Pharmacogenetics*.

Susan E. Walther, Amanda Thistle and Rebecca Blanchard, Association of SULT1A1 pharmacogenetics with cellular response to estrogens and antiestrogens, *in preparation*

CONCLUSIONS
In summary, we have made significant progress in achieving the aims set forth in this grant. Specifically we have determined the SULT1A1 and, to a lesser extent, UGT1A6 contribute to the conjugative metabolism of OHT, the active metabolite of TAM and that UGT1A9 is predominantly responsible for RAL glucuronidation. Furthermore, we have identified and determined the allele frequencies for four common UGT1A6 alleles in ethnically defined human populations and have determined genotype/phenotype relationships for those alleles in a bank of human liver tissues. We have also studied each of the four recombinant UGT1A6 allozymes and collectively our data suggest that the *2 allozyme is associated with two-fold higher activity. We have developed a cell model system to study SULT1A1 and UGT1A6 allele-specific cellular phenotypes and have implemented that system to identify allele-specific proliferative response of cells to estrogens and antiestrogens. Specifically, we have determined that cells expressing the SULT1A1*2 variant respond to those compounds significantly more than cells expressing the SULT1A1*1 allele. The mechanism for this phenotype appears to be attributable to both altered enzyme kinetics as well as altered intracellular protein stability. Approximately 30% of Caucasians and African Americans are homozygous for the SULT1A1*2 allele. These results are significant in that should these allele-specific responses also occur in tumors -- SULT1A1 genotype might be associated with clinical response to tamoxifen. That possibility will be explored in a clinical study as part of specific aim 3 of this grant.

REFERENCES

None

APPENDICES

Amended Human Subjcts Protocol

PHARMACOGENETIC FACTORS CONTRIBUTING TO VARIATION IN THE RESPONSE TO TAMOXIFEN

IRB # 00-801

Principal Investigator	Rebecca L. Blanchard, Ph.D. Associate Member, Dept of Pharmacology Fox Chase Cancer Center (215) 728-3141
Co-Investigator	Lori J. Goldstein, M.D. Director, Breast Evaluation Center Fox Chase Cancer Center
Statistician	André Rogatko, Ph.D. Director, Biostatistics Shared Facility Fox Chase Cancer Center
Coordinating Center	Protocol Management Office Fox Chase Cancer Center 7701 Burholme Avenue Philadelphia, PA 19111 Phone: (215) 728-2981 Fax: (215) 214-4029
Study Participants	Fox Chase Networks

Protocol Date	3/28/00
Amendment 1	6/8/00
Amendment 2	10/19/00
Amendment 3	11/12/01
Amendment 4	6/30/03

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Appendix I: Consent Form

Appendix II: Forms Submission Schedule, Data Collections Forms and Study Tools

Appendix III: FCCC Network Hospitals

Appendix IV: CTC Version 2.0 Grading for Anticipated Tamoxifen Side Effects

Investigator Responsibilities

Dr. Blanchard will direct and coordinate the overall project. Laboratory measurements will be performed under the direct supervision of Dr. Blanchard. Dr. Goldstein will coordinate and supervise the referral of patients to this study and the collection of clinical data. Dr. Rogatko has participated in the study design and will perform statistical analyses with regard to outcomes associated with this study.

Introduction and Background

Pharmacogenetics is the study of genetic polymorphisms and their contribution to interindividual variation in response to drugs. The application of fundamental basic pharmacogenetic principals has begun to allow for the individualization of pharmacotherapy to promote efficacious drug therapy and prevent toxic drug reactions [1-3]. The purpose of this clinical study is to determine the association between patient genotype for several drug-metabolizing genes and clinical response (time to progression) and tolerance to the antiestrogen tamoxifen (TAM). TAM has been a mainstay in the first-line treatment of metastatic breast cancer since 1985, and in 1998, it was recognized that women treated with TAM for breast cancer experienced a significant reduction in the incidence of contralateral breast cancer [4]. This observation prompted the National Surgical Adjuvant Breast and Bowel Project (NSABP) to sponsor the Breast Cancer Prevention Trial (BCPT) to evaluate the efficacy of TAM therapy as a breast cancer preventive agent in women at increased familial risk. Overall, TAM was found to reduce the incidence of breast cancer by as much as 50% and was subsequently approved as a chemopreventative agent [5]. However, recent studies suggested the superiority of aromatase inhibitors, such as anastrozole, in the first-line therapy of metastatic breast cancer [6]. Two multi-center trials of anastrozole demonstrated equivalent response rates, superior toxicity profiles, and enhanced tolerability relative to tamoxifen in advanced breast cancer therapy [7]. Thus, tamoxifen is now most commonly prescribed in the adjuvant treatment of estrogen receptor (ER) positive breast cancer of all stages and in high-risk populations with a five-year dosing regimen [8]; therefore, potentially hundreds of thousands of women will be prescribed TAM over the next few years.

TAM is hydroxylated to 4-hydroxytamoxifen (OHT) primarily via two cytochrome P450s, CYP3A4 and CYP2D6 [9,10]. OHT, while quantitatively not the major metabolite of TAM, is 100-fold more potent than TAM and is considered the active metabolite [4]. OHT is inactivated by sulfate and glucuronide conjugation reactions [11-13]. We have shown these reactions to be catalyzed by SULT1A1 (a sulfotransferase) and UGT1A6 (a glucuronosyltransferase), respectively. In the present studies, associations between genotype and both efficacious and toxic response to tamoxifen therapy will be assessed. The drug-metabolizing genes that will be evaluated include CYP2D6, CYP3A4, SULT1A1, and UGT1A6. These genes were selected because they represent pathways critical to the bioactivation and inactivation of OHT and because functionally significant polymorphisms exist within these genes [9,10,12,14-18]. Approximately 300 women who are being prescribed tamoxifen as part of their adjuvant breast cancer treatment will be recruited for this study from Fox Chase Cancer Center and Fox Chase Network Hospitals. Participation will involve the collection of two tubes of blood from which DNA will be isolated as well as the collection of medical information pertaining to the participant's clinical response to tamoxifen therapy. Patient accrual will begin

in August 2003 and continue until 300 women have been recruited. It is estimated that this will take one year. The total length of the study, including experimental procedures and clinical follow-up will be three years.

Objectives and Rationale

Response to tamoxifen therapy is known to be subject to interindividual variation. Approximately 50% of women who receive the drug for the treatment of breast cancer show a favorable clinical response including a delayed time to progression [19-21]. Hot flashes occur in approximately 46% of women taking tamoxifen (vs. 29% in those not receiving the drug) and those women are at 2.5 times greater risk for developing endometrial cancers than women who do not receive tamoxifen [5]. Furthermore, women receiving tamoxifen therapy experience vascular events 1.5 times more often than women who do not receive tamoxifen [5]. **We hypothesize that interindividual variation in the bioactivation and inactivation of OHT contributes to variation in the clinical and adverse responses to the drug, and furthermore, that genetic polymorphisms in genes involved in the metabolism of tamoxifen contribute to this variation. The scientific objective of this study is to determine if association exists between CYP2D6, CYP3A4, SULT1A1, or UGT1A6 genotype and clinical efficacy and/or toxicity of tamoxifen in the therapy of early stage (I-IIB) breast cancer.**

~~Primary Objective~~

~~To determine the association between drug-metabolizing genotype(s) and the response rate to tamoxifen (percent of responders).~~

~~Secondary~~ **Objectives**

1. To determine the association between drug-metabolizing genotype(s) and time to progression of the early stage disease.

2. To determine the association between drug-metabolizing genotype(s) and the incidence of adverse reactions and toxicity such as ocular toxicity, thromboembolic events, hot flashes, endometrial cancers, and vaginal bleeding or discharge.

Registration of Study Participants

Approximately 300 women being treated for metastatic breast cancer who are currently undergoing clinically indicated adjuvant tamoxifen therapy will be selected for participation in this study. To avoid bias against accrual of women who are non-responders to tamoxifen therapy, we will only register women who have been treated for six months or less with tamoxifen in the setting of their early stage disease. Recruitment of study participants and collection of clinical data will be managed by the Protocol Office at Fox Chase Cancer Center.

Protocol Management. The Fox Chase Cancer Center Protocol Office is an established, centralized resource that facilitates the development, conduct, quality assurance monitoring and evaluation of clinical trials. As such, this office coordinates the majority of clinical studies at Fox Chase Cancer Center. This effort includes attention to scientific, ethical, and regulatory issues, as well as objective and verifiable data management for approximately 150 individual trials that are open to patient accrual at any one time. Upon initial approval of a study by the Institutional Review Board, the protocol is assigned to one of eight teams consisting of a clinical research coordinator and a clinical research associate. Each team is organized by disease or

specialty, and the individuals serve as a resource for physicians, clinical staff, and patients. The Protocol staff is involved in direct patient/physician interactions, as well as chart review for data management to accurately depict the course of each patient on each study.

Network Participation. In addition, the Protocol Office is responsible for coordinating the research efforts of the 14 hospital systems that are affiliated with Fox Chase through the Fox Chase Network (Appendix III) to which enrollment in this protocol will be extended. Each site has a clinical research associate who serves the Protocol Office role as described above for that institution. Fox Chase Cancer Center offers a quarterly scientific dinner to which all Fox Chase Network clinicians and all clinical research associates are invited to attend. The purpose of these dinners is to educate the Network institutions about the protocols that are open for enrollment. Dr. Blanchard will present this protocol at one of these meetings once the protocol has been activated. In addition, the Fox Chase Network has two dedicated full-time Clinical Research Coordinators who serve as support for the Network sites. The Network Clinical Research Coordinators routinely conduct monitoring and auditing visits to each network site. Quarterly educational sessions are held at Fox Chase Cancer Center for all of the Clinical Research Staff at the Network sites to attend. For distant network sites, these conferences are conducted via interactive satellite video conferencing. The Protocol Office puts out a monthly newsletter to the Network Hospitals detailing the research protocols for which they are eligible to enroll patients.

Registration and Consenting Patients. The physician or the clinical research associate will explain the study to the patient, explain what participating in the study entails for the patient, and provide them with a written consent form. The consent form allows for the patient to consent separately to 1) this specific study and 2) use of their blood for future research studies. The patient will sign the consent form and return it to the physician or coordinator if they choose to participate. They will be provided with a copy of that signed form for their records. The patient is free to contact Dr. Blanchard at any time for further information about the study and her telephone number and e-mail address will be provided on the consent form.

Registration of study subjects will occur by physician referral to the Fox Chase Cancer Center Protocol Office at (215) 728-2981. The patient's physician or the institution's clinical research associate will complete the Physician/Coordinator Baseline Assessment, obtain informed consent from the patient and complete the On Study form. Upon review and confirmation of eligibility, the Fox Chase Protocol Office staff will assign the patient a specific sequence number. This will be sent back to the Network site on a confirmation form and this identifier will be placed on all future completed case report forms in lieu of the patient's name. The completed Physician/Coordinator Baseline Assessment, consent forms and On Study forms will be returned to the Protocol Office. Network participants will fax those forms to the Protocol Office at (215) 214-4029. Dr. Blanchard and Dr. Rogatko will not have access to the "On-Study" registration forms or the Physician/Coordinator Baseline Assessment as these contain patient identification information. A copy of the signed consent form will be given to the study registrant and the original will be stored at the institution administering the consent. As previously noted, Fox Chase Network auditors routinely monitor and audit those records.

Collection of Blood. After patient consent has been obtained, blood samples will be collected by the physician or designated phlebotomist. The Protocol Support Laboratory will identify each participant to the Biosample Repository using a study sequence number. The participant's clinician(s) and Protocol Office personnel will maintain patient identity, but no identifiers will be available to Fox Chase Cancer Center research personnel. It is important to note that the only affect that participation in this study will have on the treatment of study

participants is the additional collection of two tubes of blood. No modification of their current treatment will be implemented. Although available clinical data will be collected from study participants, this is data that will be generated from testing that is part of the routine care of these women. Participants will not be excluded on the basis of age (except that they must be over the age of 18), or ethnicity, although those parameters will be recorded and used as stratifiers in the data analyses. Patients will have the opportunity to consent separately for the use of their blood in 1) this research study and 2) future research studies.

Collection of Data. Clinical Research staff will collect available data from the medical records of each study participant at each patient visit. Collection of clinical data by the physician and/or clinical research associate will be performed using the data collection forms provided as Appendix II of this protocol, and should begin from the date on which the patient began tamoxifen therapy. The completed data forms will be returned to the Fox Chase Cancer Center Protocol office by faxing them to (215) 214-4029. Each site (FCCC and Network Hospitals) will retain the original copies of the data collection forms and store them in a locked filing cabinet for a minimum of five years. Drs. Blanchard, Goldstein and Rogatko will have access to the data collection forms for extraction of data directly onto an electronic database that will also contain genotype data. The electronic database will be accessible to Drs. Blanchard, Goldstein and Rogatko and will be password protected but will contain no information allowing for direct or indirect identification of the patient. The data collection forms to be used for this study do not include any patient identifiers. Clinical Research staff will use a specific sequence number to identify samples and data collection forms. The faxed Data Collection Forms will be stored in a locked file cabinet in the Protocol Office. Data collection will continue for three years and all forms will be stored for at least five years. At no time will Dr. Blanchard or Dr. Rogatko access patient records directly, and all data collection forms are devoid of patient identifiers.

Inclusion Criteria

Criteria for inclusion are:
1. Documented history of early stage (I-IIB) adenocarcinoma of the breast
2. Currently treated with adjuvant tamoxifen therapy for $\leq$ to six months at time of registration
3. Receiving no concomitant chemotherapeutic agents, radiation, or hormone therapy during tamoxifen treatment
4. ~~Evaluable disease~~ Screening laboratory tests completed within 30 days of registration |
5. Age $\geq$ 18
6. Adequate renal function (serum creatinine $\leq$ 2 or creatinine clearance $\geq$ 50 ml/min)
7. Adequate liver function (LFTs $\leq$ 2X upper limit of normal)
8. Patient must be female
9. Written patient consent

Methods

After patient consent has been obtained, approximately 20 ml (two yellow top tubes) of peripheral blood will be drawn from each participant either by the clinician or in the phlebotomy unit of the clinic. The time of blood draw is not critical because our primary objective is to isolate genomic DNA from the blood. Blood will be sent along with the appropriate Case Report Form (Lab Specimen Shipping Form) to the Protocol Support Laboratory/ Biosample Repository (PSL/BR), Room P2011, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA

19111, where it will be processed, and stored. The Lab Specimen Shipping Form only includes the patient identification number and does not include the patient's name. DNA will be isolated from each blood sample in the PSL/BR using standard extraction methods. Isolated DNA will be identified by study number only and stored in the PSL/BR facility. Blood plasma, platelets and white blood cells will also be isolated and stored at −80°C in the PSL/BR in the event that these samples might be used for future research relevant to tamoxifen metabolism. Blood samples and products will have no markings allowing the laboratory scientists to identify the study participant. From the PSL/BR, an aliquot of DNA will be sent directly to Dr. Blanchard's laboratory for genotyping analyses. Dr. Blanchard will store that aliquot of DNA in her laboratory, at 4 °C.

Clinical data will be collected at the patient's regularly scheduled clinic visit via the data collection forms and mailed or faxed to the Fox Chase Cancer Center Protocol Office, Room C121, 7701 Burholme Avenue, Philadelphia, PA 19111, or (215) 214-4029. Laboratory tests are to be drawn at the discretion of the physician and no additional tests above and beyond those tests required for the routine care of the patient are required. It is not a requirement that all of the data listed in the data collection forms to be available for participation in this study, however all available data shall be recorded. That data will be entered into a database managed by Dr. Blanchard and Dr. Rogatko and the forms stored as previously described. Data will be stored electronically as well as in hardcopy format in a database that will include the following information: participant study identification number, age, ethnicity, duration of tamoxifen therapy, other drugs administered, incidence of toxicities, rate of tumor progression, time to progression of disease, survival and genotypes.

Research laboratory procedures and experiments will be conducted under the supervision of Dr. Blanchard. Each participant will be genotyped for the genes of interest by the use of several PCR reactions and restriction enzyme-based diagnostic assays. Assays for CYP2D6 and CYP3A4 have been established and published by other investigators while the assays for the SULT1A1 and UGT1A6 have been developed in Dr. Blanchard's laboratory [22-24].

Clinical Response and Adverse Events. ~~Clinical response will be assessed by the attending physician at each visit according to standard response criteria. Those criteria are:~~

~~Complete Response (CR): Complete disappearance of all tumor lesions for at least four weeks from the date of documentation of complete response.~~

~~Partial Response (PR): Decrease by 50% or greater in the sum of the products of the two largest perpendicular diameters of all measurable lesions as determined by two consecutive observations at least four weeks apart. A decrease by 50% or greater is also required for unidimensional lesions. No lesion, measurable or not, should have progressed and no new lesions should appear.~~

~~Stable Disease (SD): Failure to observe remissions as defined above, in the absence of any progressive or new lesions, as determined by two consecutive observations at least four weeks apart.~~

~~Progressive Disease (PD): At least 25% increase in the size of any measurable or evaluable lesion and/or the appearance of any new lesions or the occurrence of a~~

~~malignant pleural effusion or ascites. In the case of serum tumor marker evaluations, at least 50% increase over any nadir value will constitute progressive disease, and/or in association with the appearance of any new lesions or the occurrence of a malignant pleural effusion or ascites.~~

~~In addition, non-measurable but evaluable disease, (e.g., pleural effusion, ascites, and serum tumor marker evaluations) will not be considered in assessment of response status except in the instance of progressive disease defined as above.~~

The time to progression of the disease will be measured by the appearance of new primary or metastatic lesions. In the case of serum tumor marker evaluations, at least a 50% increase over any nadir value will constitute progressive disease in the presence or absence of any new primary or metastatic lesions. Adverse events that are possibly related to tamoxifen therapy (including ocular toxicity, hot flashes, thromboembolic events, endometrial cancers, and vaginal bleeding or discharge) shall be recorded on data collection forms.

Statistics

Power Analysis. The primary objective of the study is to determine whether there is an association between CYP2D6, CYP3A4, SULT1A1 or UGT1A6 genotypes and clinical response. The association between the homozygote recessive genotype and clinical response will be tested in each allele system. It was assumed that 50% of patients will have a favorable response to the treatment and that the genotypic frequency of the recessive homozygote is 9% in the positive clinical response group. Table 1 shows genotypic frequencies in the negative clinical outcome group that would allow rejection of the null hypothesis for each system, assuming 80% power and 5% significance in a two-tailed test. For small genotypic frequencies (e.g., .01 and .07), the minimum detectable difference that would provide adequate power is greater than the underlying genotypic frequency. This implies that a two-sided test will not have adequate power in one direction. In this case, a one-tailed test was applied.

Table 1. Minimum detectable differences to test the association between presence or absence of cancer in individuals and genotypic frequencies for different gene systems, assuming 80% power and 5% significance in a one or two-tailed test. The sample consists of 150 individuals with a favorable response to the treatment and 150 in the negative clinical outcome group.

System	Genotypic frequency in the positive clinical outcome group	Genotypic frequencies in the negative clinical outcome group that would allow rejection of the null hypothesis	One or two-tailed test
G_1	0.1	<0.01 >0.203	Two
G_2	0.07	>0.15	One
$G_{3,1}$	0.01	>0.045	One
$G_{3,2}$	0.28	<0.138 >0.428	Two

Statistical Analysis.   Binary logistic regression techniques will be used to test the hypotheses previously described and select the significant predictors (genotypes) for each system for presence for negative or positive clinical outcome Interaction between predictors will be also considered. Maximum likelihood will be used to estimate the parameters for all the logistic regression models. Hypotheses will be tested using likelihood ratio methods. Hardy-Weinberg equilibrium will be evaluated with methods presented by Rogatko *et al.* [25] and software described in Rogatko and Slifker [26].

Benefits and Risks
    The study participants will not benefit directly from this research and the only health risks are those posed by blood collection. Those risks include bruising at the venipuncture site, minor pain, and, rarely, fainting. To minimize these risks, blood collection will be performed only by clinicians or trained phlebotomists. If any unexpected adverse effects related only to the blood draw procedure are experienced, they will be immediately reported to the USAMRMC Deputy Chief of Staff for Regulatory Compliance and Quality by calling (301) 619-2165. A written report will follow within three days and sent to:
        US Army Medical Research and Materiel Command
        ATTN: MCMR-RCQ-HR
        504 Scott St
        Fort Detrick, MD 21702-5012

Signature of Principal Investigator
    I have read the foregoing protocol and agree to conduct the study as outlined herein.

_____   _____

Signature of Principal Investigator   Date

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